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Energy metabolism and glycolysis in human placental trophoblast cells during differentiation

Bridget E. Bax [1], David L. Bloxam [*]

Reproductive Biology Laboratory, Royal Postgraduate Medical School Institute of Obstetrics and Gynaecology, Queen Charlotte's and Chelsea Hospital, Goldhawk Road, London W6 0XG, United Kingdom

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Abstract

Energy metabolism and glycolysis of normal human term placental trophoblast in two-sided culture was investigated during differentiation from cytotrophoblast to syncytiotrophoblast, because glycogen metabolism is abnormal in several trophoblast related pregnancy diseases, including pre-eclampsia. After initial recovery of energy and cytoplasmic $NADH/NAD^+$ redox by 24 h of culture, measures of cellular energy state, [ATP], [ADP], [ATP]/[ADP] ratio, ([ATP] + [ADP] + [AMP]), [ATP]/([ATP] + [ADP] + [AMP]) and energy charge remained essentially constant until 72 h, despite periods of increased energy turnover. At 24 h there was a burst of glycogenolysis, and glycolysis indicated by increased lactate production, which coincided with formation of syncytium. Subsequently, there was no resynthesis nor further breakdown of glycogen. At 48 h, oxygen consumption temporarily increased substantially, without increased glycolysis, during functional differentiation of the syncytiotrophoblast. Glucose uptake was constant and largely from the basal (in vivo fetal facing) side. Lactate output into the basal fetal medium was twice as fast as that into the microvillous (maternal) medium, and oxygen uptake was also asymmetrical. The results show that before and after differentiation substantial relatively constant aerobic glycolysis occurs, but that during increased energy demand cytotrophoblast depends on both glycolytic and aerobic energy production whereas syncytiotrophoblast relies on aerobic metabolism.

Keywords: Cultured syncytiotrophoblast; Placental trophoblast energy metabolism; Placental trophoblast glycolysis; Trophoblast glycogenolysis; Trophoblast adenine nucleotide metabolism; Trophoblast differentiation

1. Introduction

Syncytiotrophoblast is the epithelial cell layer that mediates the specific maternal-fetal nutrient transfer, hormone synthetic and secretory processes of the human placenta, all of which require large amounts of energy. It is derived from the stem cell, cytotrophoblast, which can differentiate into various different placental cell types with different functions (e.g., [1]). There is evidence of abnormal carbohydrate metabolism as well as aberrant differentiation in several trophoblast-related pregnancy diseases, including pre-eclampsia, choriocarcinoma and hydatidiform mole (e.g., [2–5]).

We have recently described methods for the isolation and culture of human near term placental cy-

* Corresponding author. Fax: (44) (181) 944 1977.
[1] Present address: Department of Child Health, St. George's Hospital Medical School, London SW17 0RE, UK.

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totrophoblast cells. In our conditions, these spontaneously differentiate during culture into a polarised syncytial monolayer of epithelial syncytiotrophoblast [6,7]. We can now culture these cells on a permeable membrane with medium on both sides [7,8]. As a first step to understanding trophoblast energy metabolism and its relationships with trophoblast differentiation we have investigated changes in glycolysis and energy metabolism with time during the differentiation of these cells from cytotrophoblast to syncytiotrophoblast, including directional aspects of uptake or release of glucose, lactate and oxygen across the microvillous (in vivo maternal facing) and basal (in vivo fetal facing) surfaces of the cells.

2. Materials and methods

2.1. Trophoblast isolation and two-sided culture

2.1.1. Preparation of cells

Human cytotrophoblast cells were isolated from villous tissue obtained from placentas delivered by elective caesarean section at 38–39 weeks of estimated gestation for reasons not indicating any placental abnormality or disease. For the investigation of cellular consumption of glucose and oxygen, and output of lactate, the method was as previously described in detail [6,8]. Briefly, 2–4 cm^3 portions of dissected placental villous tissue, collected in phosphate buffered saline (PBS; 153 mmol/l Na^+, 142 mmol/l Cl^-, 10 mmol/l HPO_4^-, 0.9 mmol/l Ca^{2+}, 0.5 mmol/l Mg^{2+}, pH 7.4) supplemented with 100 000 U/l penicillin, 100 mg/l streptomycin, 2 mmol/l L-glutamine, 5 mmol/l glucose and 0.3 mmol/l pyruvate (supplemented PBS), additionally containing 1000 U/l lithium heparin, were rinsed then shaken in more supplemented PBS without heparin at 37°C for 15 min. Approximately 20 g of the tissue was then cut into 1–2 cm^3 pieces and incubated with shaking at 37°C with supplemented PBS containing 20 mg/ml protease XV (Sigma Chemical, Poole, UK) and an aqueous extract (3 mg/ml) of porcine pancreas acetone powder prepared by the method of Henry [9]. When aggregates of cells could be detected in the buffer by light microscopy (usually 25–35 min) the pieces of tissue were removed and shaken gently for 60 s with an equal vol of fresh supplemented PBS containing acetone powder extract (equivalent to 0.6 mg powder/ml), then rinsed in more supplemented PBS with acetone powder extract. The tissue residue was discarded and the two resulting cell suspensions were pooled, shaken gently to disperse cell aggregates and filtered consecutively through two and three layers of sterile 12 ply surgical gauze to remove debris. The cells were centrifuged (90 s at 100 *g*) and washed with Medium 199 (Flow Laboratories, Rickmansworth, UK) buffered at pH 7.4 with 20 mmol/l Hepes, supplemented with 100 000 U/l penicillin, 100 mg/l streptomycin and 2 mmol/l L-glutamine, before plating on cell-free amniotic membrane in the same medium with 5% (vol/vol) pooled human umbilical cord serum. The yield of cells was typically $50–60 \cdot 10^6$ per 20 cm^3 of original tissue, and they were characterised immunocytochemically as consisting of at least 99% villous trophoblast essentially free from other cell types present in placenta [6,8].

2.1.2. Preparation of cell-free amniotic membrane

Amnion was separated from chorion of placentas obtained from elective caesarean section. Only *amnion reflecta* (the area not adjacent to the placenta and umbilical cord) was used. This was washed in supplemented PBS, then the associated cells were lysed by incubating in 20 mmol/l ammonium hydroxide under sterile conditions for 10–15 min at room temperature. The lysed amnion epithelial cells on one side and the gelatinous spongy layer [10] on the other were removed by gentle wiping, and the resulting cell-free amniotic membrane was washed extensively in supplemented PBS [8].

2.1.3. Two-sided culture

As described in detail elsewhere [8], pieces of the washed cell-free amniotic membrane were stretched over one (lower) end of 14 mm internal diameter, 25 mm long, polypropylene cylinders, with the compact layer [10], previously occupied by the amnion epithelium, uppermost, and held by silicone rubber 'O' rings. These units were suspended in supplemented Medium 199 with 5% (vol/vol) human umbilical cord serum (culture medium) contained in the 24 mm diameter wells of standard 12 well cell culture plates (Linbro, Flow Laboratories). Freshly isolated cytotrophoblast cells were plated onto the membrane surface

at a density of $1 \cdot 10^6$ cells per cylinder, in the same culture medium (see [8]). The cultures were maintained in a humidified atmosphere at 37°C. The culture medium volumes were, in the upper compartment 1.5 ml and in the lower compartment 3.5 ml. The media in both compartments were changed after several hours to remove the blood cells and non viable trophoblast cells which do not adhere, and once a day thereafter. For the measurements of cellular metabolite concentrations, this standard system was scaled up 16 fold to provide enough tissue for all the metabolites to be assayed in a single culture preparation.

The trophoblast cells during culture have been extensively characterised morphologically, immunocytochemically and biochemically [6–8]. It has been found that in this two-sided culture system, the cells, initially villous cytotrophoblast, spontaneously differentiate over the course of two days into a continuous unicellular polarised epithelial sheet so far indistinguishable from villous syncyntiotrophoblast in vivo [6–8]. The time course of differentiation was followed by light microscopy in the present experiments and was identical with that reported previously [8]. Immunocytochemical studies showed that throughout the culture period the cells were more than 99% trophoblast, and that no cells normally associated with untreated amnion (amnion cells, macrophages and fibroblasts) were present in the cell culture or supporting amniotic membrane [8]. Essentially all the cells remained viable during the culture period up to 72 h, judged by Trypan Blue exclusion and DNA content.

2.2. Assays

Assays of metabolites were carried out in cell extracts after 0, 6, 24, 48, 72 and 96 h of culture, and in the culture media after 0, 24, 48, 72 and 96 h. Samples of medium both from the upper, 'microvillous', compartment (i.e., that which had been in contact with the microvillous, or apical, surface of the trophoblast cell layer) and from the lower, 'basal', compartment (in contact with the basal cell surface) were stored at -20°C. For assay they were deproteinised with an equal volume of 1.5 mol/l perchloric acid, centrifuged, and the supernatant neutralised with KOH/K_2HPO_4 as described previously [11]. The trophoblast cell layers attached to the amniotic membrane were rapidly cut from the culture cylinders with a scalpel, shaken to remove excess medium, and immediately dropped into and stored in liquid N_2. For assay, the frozen tissue samples were deproteinised by homogenisation in 3 volumes of ice cold 1.5 mol/l $HClO_4$, centrifuged and neutralised as for the medium samples.

Cellular concentrations of ATP, ADP, AMP and glycogen, and cell and medium concentrations of glucose, lactate and pyruvate were measured in the neutralised perchloric acid extracts or tissue whole homogenates (as appropriate) by enzymatic methods involving the measurement of changes in NADH either spectrophotometrically (Cecil Instruments CE5095 High Performance Scanning Spectrophotometer) or fluorometrically (Perkin Elmer 3000 fluorescence spectrometer) as described in detail elsewhere [11]. Cellular DNA concentrations were measured fluorometrically using Hoechst 33258 reagent (2-[2-(4-hydroxyphenyl)-6-benzimidazolyl]-6-(1-methyl-4-piperazyl)-benzimidazol · 3HCl) as described by Labarca and Paigen [12] in cell samples on amniotic membrane ultrasonicated (Soniprobe 1130A, Dawe Instruments, UK) in PBS. Weights of cell samples were determined after the DNA measurements from the previously derived relationship between cell weight and DNA content, corrected for extracellular fluid with ^{14}C-labeled inulin (inulin-carboxyl [^{14}C], DuPont, Hertfordshire, UK). By calculating metabolite concentrations from values relative to tissue DNA content, any loss of non viable cells during culture was corrected for. Cellular metabolite net uptakes and outputs were determined for 24-h periods (see Fig. 4 and Fig. 5) from concentration changes in the media on each side of the trophoblast layer. Fresh media were added to the two compartments for each 24-h period. Preliminary experiments were done which indicated that oxygen was present in excess of cell requirements throughout the 24-h incubation periods. Oxygen consumption was measured in the same way during 8-h periods (Fig. 7), using a Radiometer ABL2 blood gas analyser, in cultures in which O_2 was prevented from entering or escaping by adding a layer of paraffin to the media surfaces. Initial experiments incubating trophoblast cells to different degrees of O_2 depletion showed that in the presence of paraffin oxygen sup-

ply did not become limiting to its utilisation until 12 h or later.

2.3. Light and electron microscopy

Light microscopy and transmission electron microscopy were carried out as described in detail elsewhere [8]. We are very grateful to Dr. T.A. Ryder and Margaret A. Mobberley, Electron Microscopy Unit, Queen Charlotte's and Chelsea Hospital, for this part of the work.

3. Results and discussion

The results in Fig. 1, Fig. 2, Fig. 3, Fig. 4, Fig. 5 and Fig. 6 show that marked changes in energy metabolism and glycolysis occurred at specific times during the culture period when the isolated placental cytotrophoblast cells were differentiating into syncytiotrophoblast. These changes were not due to a degeneration of the cells because their energy state, judged by ATP and ADP concentrations, ATP/ADP ratio and energy charge [13] (Fig. 1), either increased or remained steady up to 72 h of culture. Between 72 and 96 h of culture, however, there was evidence of a decline of cellular energy state, by the same criteria, which corresponded with morphological evidence of a slow deterioration of the condition of the cells [6] after 72 h.

3.1. 0–24 h of culture

During the initial hours of culture there was a very marked increase in the indicators of cellular energy level ATP, ATP/ADP, energy charge (Fig. 1) and ATP/AMP (not shown). They all reached steady-state plateau values by 24 h, very similar to estimated in vivo values in whole placenta [11]. This presumably reflects rapid recovery of the cells from the trauma and perhaps hypoxia during the isolation procedures. This interpretation is supported by the initially relatively high lactate and low glycogen levels (Fig. 2, Fig. 3 and Fig. 7); and by the relatively reduced state of the cytoplasmic $NADH/NAD^+$ reduction-oxidation couple, indicated by the lactate/pyruvate concentration ratio [14] (Fig. 3), which is known to become more reduced during hypoxia [11,15]. The

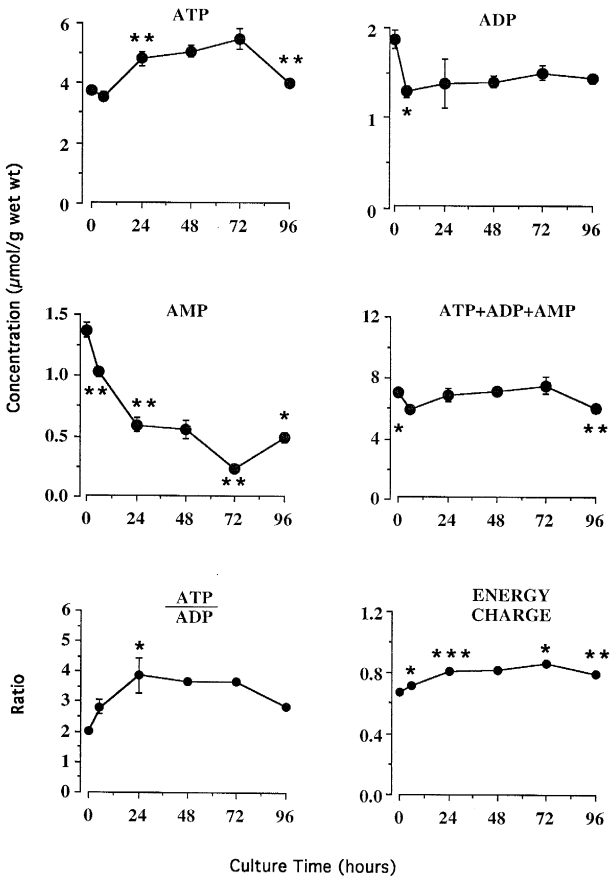

Fig. 1. Cellular concentrations of ATP, ADP, AMP, and their sum, ATP/ADP concentration ratios, and energy charge (ATP $+\frac{1}{2}$ADP)/(ATP + ADP + AMP) [13], at different times during two-sided trophoblast culture. Results are the mean values ± S.E.M. of preparations from four different placentas. Culture conditions are described in Section 2. Asterisks indicate the significance of differences between the value at a particular time and that at the previous time; i.e., the significance of the change from one time to the next (analysis of variance followed by *t*-test): *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

high AMP concentration (Fig. 1) and low ATP/AMP ratio (not shown) found in the cells at zero time, also occur in placenta during cellular ischaemia [11,15]. Alongside the subsequent improvement of energy state during the first 24 h of culture, there was a rapid fall of AMP concentration and rise of ATP/AMP ratio (important in the regulation of energy metabolism, particularly in anoxia [16,17], to plateau levels, falls of lactate concentration and lactate/pyruvate ratio – the latter to values similar to those estimated for whole placenta in vivo [11] – and a steady synthesis of glycogen to concentrations

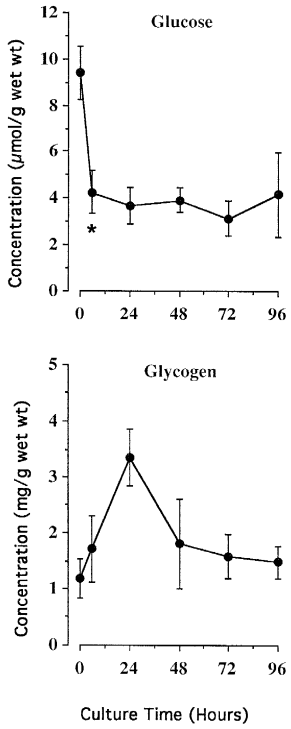

Fig. 2. Cellular concentrations of glucose and glycogen at different times during two-sided trophoblast culture. Data are from the same cell preparations as those in Fig. 1. Other details as for Fig. 1.

reaching a maximum at 24 h (Fig. 2). Further, as the ATP/ADP ratio rose to reach its plateau (Fig. 1), the rate of oxygen consumption fell (Fig. 6). All these findings confirm the picture of an initial energy and metabolic recovery towards a steady state, at least in part brought about by aerobic metabolism.

The sum of the adenine nucleotides, however, was not low at zero time (Fig. 1) suggesting that there had been no overall catabolism of the adenine nucleotides during cell isolation, which would have indicated more severe cellular injury. There was, on the other hand, a small and short-lived but statistically significant fall in total adenine nucleotide concentration in the first 6 h after the start of culture (Fig. 1). This might be due to transient injury following re-availability of oxygen, analogous to reperfusion injury that can occur in other tissues after ischaemia (e.g., [18]).

Although the initially low glycogen and raised cellular lactate concentrations imply that glycogen had broken down for energy production via the anaerobic glycolytic pathway during the isolation procedure, surprisingly, high cellular free glucose concentrations were also found at zero time of culture (Fig. 2), much higher than those of the culture medium on each side (5.4 mmol/l). Similar high free glucose concentrations have been found in freshly isolated human early pregnancy, but not term, placenta slices [19], and there is evidence from in vitro studies that early pregnancy human placenta can produce glucose [20]. Early placenta contains a higher proportion of cytotrophoblast cells than later [21], and primitive cytotrophoblast (in contrast to syncytiotrophoblast) and Langans cells of early placenta do contain particularly large amounts of glycogen [21]. This suggests that during adverse conditions villous cytotrophoblast glycogen can be converted to free glucose for use by other, possibly developing embryonic, cells. There is

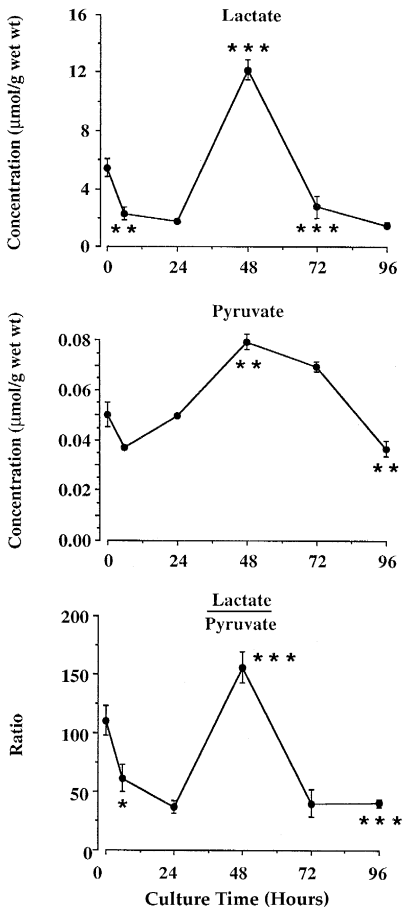

Fig. 3. Cellular concentrations of lactate and pyruvate, and the lactate/pyruvate concentration ratios at different times during two-sided trophoblast culture. Details as for Fig. 2.

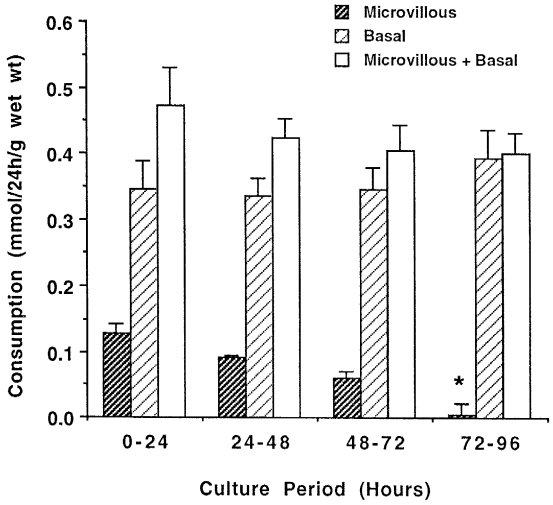

Fig. 4. Rates of glucose consumption by trophoblast cells at different time periods during two-sided culture: uptake from the microvillous compartment, uptake from the basal compartment, and total uptake (microvillous plus basal). Medium glucose concentration on both sides at the start of each time period was 5.40 ± 0.32 (S.E.M.) μmol/ml. Bars indicate S.E.M. Other details as for Fig. 2. Asterisks indicate the significance of the differences between the value for a particular time period and that for the previous time period; i.e., the change from one time period to the next: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. The rates of uptake from the microvillous compartment decreased significantly with time ($P = 0.0001$, analysis of variance). The rates of uptake from the basal compartment were greater than those from the microvillous compartment ($P < 0.0001$, two-way analysis of variance).

evidence for the presence of glucose-6-phosphatase, necessary for the conversion of glucose-6-phosphate to glucose, in human placenta (e.g., [22]), although contrary evidence has also been reported (e.g., [23]).

3.2. 24–48 h of culture

Between 24 and 48h there was a sudden rapid burst of glycolysis, indicated by net breakdown of glycogen (Fig. 2 and Fig. 7), and both cellular accumulation and increased rate of release of lactate, the latter mainly from the basal side of the cell layer (Fig. 3 and Fig. 5). However, only 14% of the increased lactate production could be accounted for by the glycogen breakdown. Despite a concomitant increase of cellular pyruvate concentration, this was accompanied by a dramatic increase of lactate/pyruvate ratio (Fig. 3). The large reduction of the cytoplasmic $NADH/NAD^+$ redox couple indicated by this lactate/pyruvate ratio change was not apparently due to hypoxia as there was no change of oxygen availability, but was probably a result of the accelerated glycolysis at the glyceraldehyde-3-phosphate dehydrogenase step which converts NAD^+ to NADH.

As the steady state cellular ATP and ADP concentrations remained constant during this period of enhanced ATP synthesis, this burst of glycolysis, as well as the enhanced aerobic metabolism (Fig. 6), presumably maintained cellular energy during increased ATP utilisation. This time period precisely coincides with the time of loss of membranes between the cytotrophoblast cells to form a syncytium, morphological changes to the nuclei and the development of placental alkaline phosphatase expression ([8], and see Section 2), all characteristic of syncytiotrophoblast formation [6,7]. This process probably requires additional expenditure of energy because it involves new protein synthesis and reorganisation of the cytoskeleton.

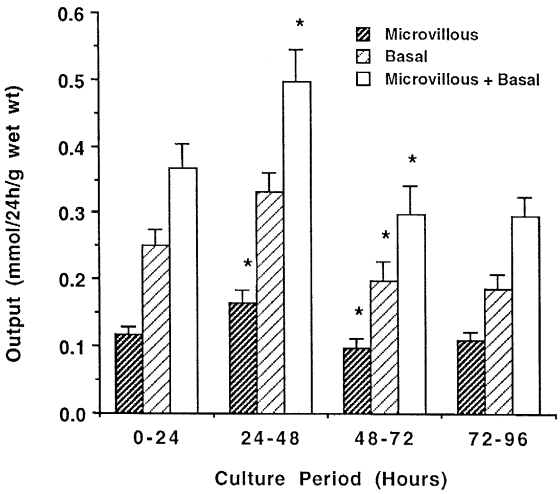

Fig. 5. Rates of lactate output from trophoblast cells at different time periods during two-sided culture: output into the microvillous compartment, output into the basal compartment, and total output (microvillous plus basal). Medium lactate concentration on both sides at the start of each time period was 0.2 ± 0.0 μmol/ml. Other details as for Fig. 4. The rates of output into the basal compartment were significantly greater than those into the microvillous compartment ($P < 0.0001$, two-way analysis of variance).

3.3. 48–72 h of culture

After 48 h, net glycogenolysis ceased and cellular lactate concentration, lactate output, and the lactate/pyruvate ratio returned to the previous (24 h) steady state levels (Fig. 2, Fig. 3 and Fig. 5), reflecting a cessation of the glycolytic burst. Nevertheless, glycogen was not redeposited thereafter, despite the sharply reduced AMP concentration (Fig. 1) and dramatically increased ATP/AMP ratio (not shown) [17]; and lactate output remained constant (Fig. 5), even during the following period of increased energy turnover. This is consistent with the relatively low glycogen concentration in syncytiotrophoblast in vivo [21] and in term placenta [11,20] which contains mostly syncytiotrophoblast, and the relative lack of response of glycolysis and glycogen levels to anoxia or ischaemia in term placenta [11,15]. Thus, whilst lactate concentrations and output and lactate/pyruvate ratio were falling, a large increase of oxygen consumption between 48 and 64 h (Fig. 6) indicated a second period of increased energy production, this time fuelled largely by enhanced aerobic oxidative metabolism. This corresponded to the time of development of hCG secretion [8], and formation of abundant endoplasmic reticulum and secretory structures seen by electron microscopy, apparently during biochemical/functional differentiation [6].

3.4. Energy metabolism and cytotrophoblast differentiation

The results show that during culture glycolysis and energy metabolism in cytotrophoblast is different from that in syncytiotrophoblast. Although compared with other human tissues there is a high rate of basal aerobic glycolysis throughout the culture period, additional energy demand seems to be met by glycogenolysis and glycolysis as well as aerobic metabolism in cytotrophoblast, whereas oxidative metabolism not fuelled by glycogenolysis is more

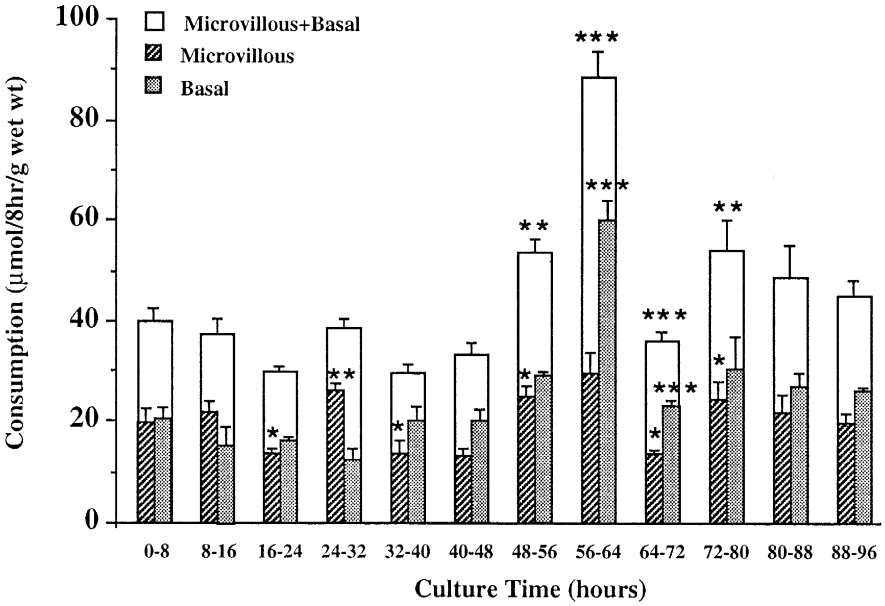

Fig. 6. Rates of oxygen consumption by trophoblast cells at different time periods during two-sided culture: uptake from the microvillous compartment, uptake from the basal compartment, and total uptake (microvillous plus basal). Medium oxygen concentrations on both sides at the start of each time period ranged from 233 ± 0.0 to 238 ± 0.8 nmol/ml. Other details as for Fig. 4. The fall in rate of total oxygen consumption between 0–8 and 16–24 h was significant ($P = 0.021$, analysis of variance). The increase in rate of total oxygen consumption between 16–24 h and 24–32 h and the subsequent fall at 32–40 h were significant when the differences were evaluated by conventional t-test ($P < 0.01$, < 0.05 respectively). The rates of total oxygen consumption from 72 h to 96 h were significantly greater than those from 0 to 24 h ($P < 0.001$). Between 0 and 32 h rates of oxygen consumption tended to be greater from the microvillous than from the basal side ($P = 0.003$), whereas between 32 and 96 h they were greater from the basal side ($P < 0.0001$, two-way analysis of variance).

important after differentiation. Thus, energy metabolism appears to differentiate along with morphological and functional differentiation. It is also possible that the mode of energy generation is specifically linked with the differentiational process occurring. For example, our results, including the electron microscopic findings (Fig. 7) are consistent with the possibility that the formation of syncytium is specifically associated with glycogen breakdown. This could explain the finding of Schmon et al. [24] of lack of glycogenolysis in cultured trophoblast cells which developed hormone secretory properties of syncytiotrophoblast, but did not fuse to form a syncytium. There is evidence that glycolytic enzymes are associ-

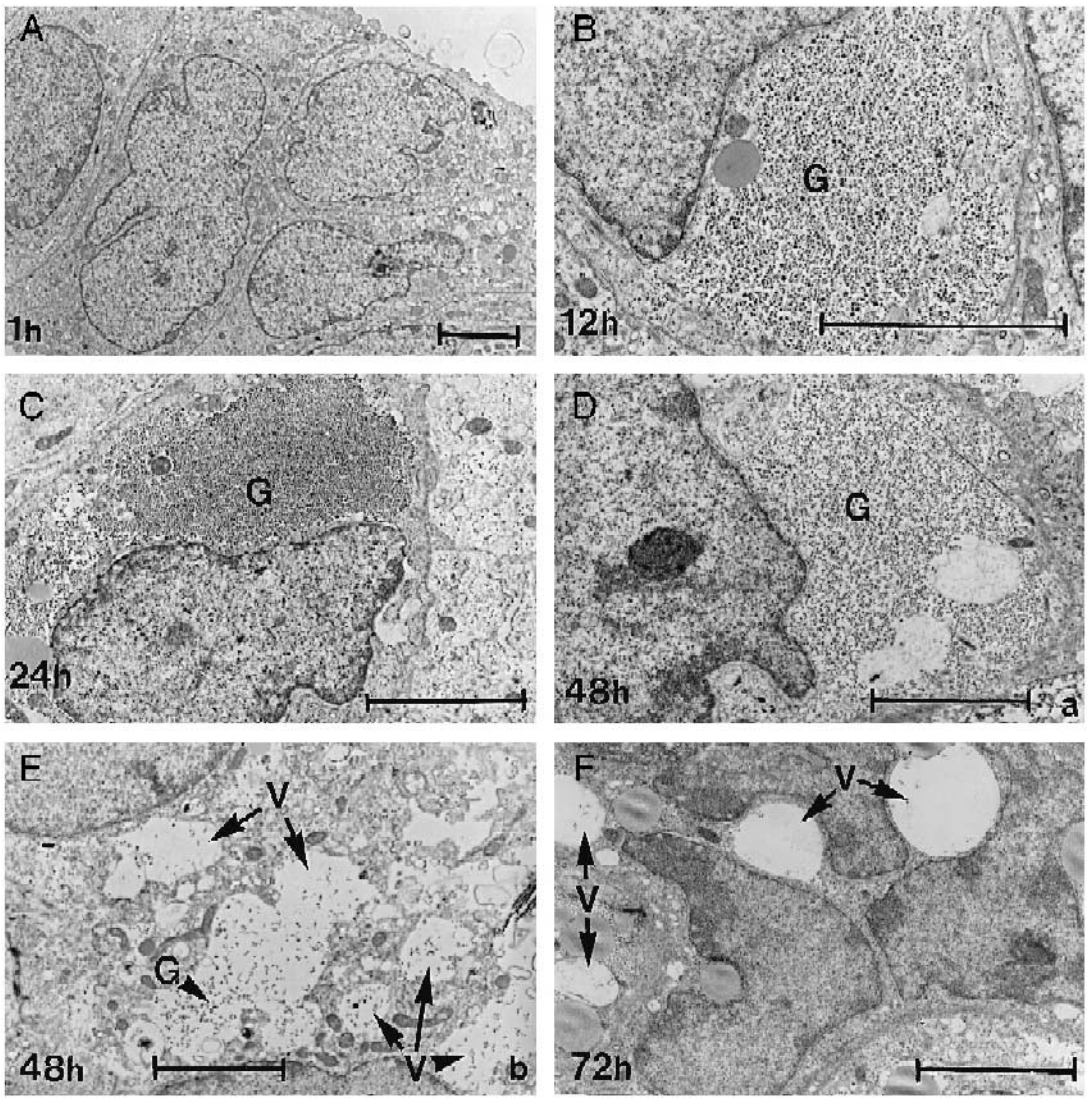

Fig. 7. Transmission electron micrographs showing presence or absence of glycogen deposits in trophoblast cells at different times during two-sided culture. When present, glycogen was found largely associated with the nuclei. 1 h: glycogen granules are absent; 12 and 24 h: glycogen granules (G) are abundant; 48 h: (a) abundant glycogen granules in the few remaining undifferentiated (cytotrophoblast) cells, (b) sparse glycogen granules in partially differentiated syncytial cells (without cell membranes between nuclei – i.e., syncytial – but before functional differentiation [6]); 72 h: little or no visible glycogen deposits in fully differentiated (syncytiotrophoblast) cells. Note presence at 48 h and 72 h of partially empty or empty vacuoles (V), presumably previously fully occupied by glycogen. Bar represents 4 μm. For descriptions of other morphological changes during cytotrophoblast differentiation and their time courses during two-sided culture, see [6] and [8], respectively.

ated with actin filaments of the cytoskeleton of cells possibly providing a local energy supply for cytoskeletal functions [25–27]. Cytoskeletal reorganisation is presumably a fundamental part of differentiation in general [28], and of syncytium formation in particular. These findings support the idea that abnormal cytotrophoblast differentiation (such as may occur in pre-eclampsia) could be accompanied by abnormal glycogen metabolism.

3.5. Asymmetry of trophoblast energy metabolism

Glucose consumption by the placenta is extremely rapid [15,29] and this affects the availability of glucose in the fetus. Recently, it has been discovered that in the pregnant sheep in vivo a substantial proportion of glucose taken up by the placenta is derived from the fetal glucose pool rather than the maternal [29,30]. Our findings indicate that, likewise, most of the glucose consumed by the two-sided cultured human placental trophoblast is taken up from the basal (in vivo fetal) compartment. Furthermore, this is an inherent property of the cell layer, not dependent upon fetal or maternal hormonal or other controls.

Earlier it had been demonstrated that the lactate produced from glucose in the sheep placenta in vivo is at least in part released into the fetal circulation and used by the fetus [31]. It is not clear whether the same is true in the human. The results reported here show that when the concentrations of glucose were equal on both sides of the trophoblast cell layer more lactate was released on the fetal than the maternal side (Fig. 5). The actual rates of release in vivo would, of course, depend on the lactate and possibly the glucose concentrations on each side, the presence of the stromal and capillary endothelial cells on the fetal side, and possible maternal and fetal regulation. There was also asymmetry of oxygen uptake by the trophoblast layer; the increase of oxygen consumption between 24 and 32 h being entirely accounted for by increased uptake on the microvillus side, whilst that between 56 and 64 h by increased uptake on the basal side (Fig. 6). In each case the asymmetry was, if anything, underestimated because of equilibrative flux across the cell layer.

The asymmetry of glucose uptake observed is unlikely to be due to asymmetrical distribution of glucose transporters on the microvillous and basal membranes. Apparent K_m and V_{max} values per mg of protein are similar on the two isolated membranes [32,33]. Therefore in the human glucose transport capacity is likely to be greater on the microvillous, not the basal, side because of its greater surface area [33]. Also, as the average intracellular glucose concentrations are the same as in the media on both sides (Fig. 2), inward transport is unlikely to be limiting to glucose metabolism. On the other hand in view of the probable intracellular compartmentalisation of glycolysis and energy metabolism in cells [26–28] alluded to above, the asymmetrical uptake of glucose despite trans-syncytiotrophoblast equilibration can be explained if the steady state concentration of glucose in the intracellular pool available for transport out on the basal side is lower than that transported out on the microvillous side. This would be the case if, for example, glucose breakdown (to lactate and/or aerobically) occurred largely close to the basal cell membrane. This could also explain the asymmetrical lactate output and oxygen consumption observed.

3.6. Aerobic lactate production

The production of lactate in fully aerobic conditions, which is characteristic of human placenta [11,15,34], was also observed here in pure trophoblast. The association of glycolysis with the cytoskeleton, and/or the reported linkage of glycolysis with active membrane transport in placenta [35] and vascular smooth muscle [36], or membrane integrity in fibroblasts [37] are of interest here because both cytoskeleton and membrane functions must be particularly active in syncytiotrophoblast: the cytoskeleton in maintaining the sheet-like shape of the syncytium and also the microvilli, and membrane transport for maternal-fetal placental transfer. It is conceivable that compartmentalised generation of glycolytic energy for these processes explains the continuous substantial aerobic lactate production by human term placenta, despite lack of marked glycogenolytic/glycolytic response to ischaemia or hypoxia.

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